# INHIBITORS OF C-JUN N-TERMINAL KINASES (JNK)

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of co-pending International Application PCT/US00/15248, filed June 2, 2000, which claims priority of United States provisional patent application 60/237,523, filed June 3, 1999.

## TECHNICAL FIELD OF INVENTION

The present invention related to inhibitors of c-Jun N-terminal kinases (JNK), which are members of the mitogen-activated protein (MAP) kinase family. There are a number of different genes and isoforms which encode JNKs. Members of the JNK family regulate signal transduction in response to environmental stress and proinflammatory cytokines and have been implicated to have a role in mediating a number of different. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

## BACKGROUND OF THE INVENTION

Mammalian cells response to extracellular stimuli by activating signaling cascades that are mediated by members of the mitogen-activated protein (MAP) kinase family, which include the extracellular signal regulated kinases (ERKs), the p38 MAP kinases and the c-Jun N-terminal kinases (JNKs). MAP kinases (MAPKs) are activated by a variety of signals including growth factors, cytokines, UV radiation, and stress-inducing agents. MAPKs are serine/theronine kinases and their activation occur by dual phosphorylation of threonine and

tyrosine at the Thr-X-Tyr segment in the activation loop. MAPKs phosphorylate various substrates including transcription factors, which in turn regulate the expression of specific sets of genes and thus mediate a specific response to the stimulus.

One particularly interesting kinase family are the c-Jun $NH_2$-terminal protein kinases, also known as JNKs. Three distinct genes, JNK1, JNK2, JNK3 have been identified and at least ten different splicing isoforms of JNKs exist in mammalian cells [Gupta et al., EMBO J., 15:2760-70 (1996)]. Members of the JNK family are activated by proinflammatory cytokines, such as tumor necrosis factor-$\alpha$ (TNF$\alpha$) and interleukin-1 $\beta$ (IL-1$\beta$), as well as by environmental stress, including anisomycin, UV irradiation, hypoxia, and osmotic shock [Minden et al., Biochemica et Biophysica Acta, 1333:F85-F104 (1997)].

The down-stream substrates of JNKs include transcription factors c-Jun, ATF-2, Elk1, p53 and a cell death domain protein (DENN) [Zhang et al. Proc. Natl. Acad. Sci. USA, 95:2586-91 (1998)]. Each JNK isoform binds to these substrates with different affinities, suggesting a regulation of signaling pathways by substrate specificity of different JNKs *in vivo* (Gupta et al., *supra*).

JNKs, along with other MAPKs, have been implicated in having a role in mediating cellular response to cancer, thrombin-induced platelet aggregation, immunodeficiency disorders, autoimmune diseases, cell death, allergies, osteoporosis and heart disease. The therapeutic targets related to activation of the JNK pathway include chronic myelogenous leukemia (CML),

rheumatoid arthritis, asthma, osteoarthritis, ischemia, cancer and neurodegenerative diseases.

Several reports have detailed the importance of JNK activation associated with liver disease or episodes of hepatic ischemia [Nat. Genet. 21:326-9 (1999); FEBS Lett. 420:201-4 (1997); J. Clin. Invest. 102:1942-50 (1998); Hepatology 28:1022-30 (1998)]. Therefore, inhibitors of JNK may be useful to treat various hepatic disorders.

A role for JNK in cardiovascular disease such as myocardial infarction or congestive heart failure has also been reported as it has been shown JNK mediates hypertrophic responses to various forms of cardiac stress [Circ. Res. 83:167-78 (1998); Circulation 97:1731-7 (1998); J. Biol. Chem. 272:28050-6 (1997); Circ. Res. 79:162-73 (1996); Circ. Res. 78:947-53 (1996); J. Clin. Invest. 97:508-14 (1996)].

It has been demonstrated that the JNK cascade also plays a role in T-cell activation, including activation of the IL-2 promoter. Thus, inhibitors of JNK may have therapeutic value in altering pathologic immune responses [J. Immunol. 162:3176-87 (1999); Eur. J. Immunol. 28:3867-77 (1998); J. Exp. Med. 186:941-53 (1997); Eur. J. Immunol. 26:989-94 (1996)].

A role for JNK activation in various cancers has also been established, suggesting the potential use of JNK inhibitors in cancer. For example, constitutively activated JNK is associated with HTLV-1 mediated tumorigenesis [Oncogene 13:135-42 (1996)]. JNK may play a role in Kaposi's sarcoma (KS) because it is thought that the proliferative effects of bFGF and OSM on KS cells are mediated by their activation of the JNK signaling pathway

[J. Clin. Invest. 99:1798-804 (1997)]. Other proliferative effects of other cytokines implicated in KS proliferation, such as vascular endothelial growth factor (VEGF), IL-6 and TNF$\alpha$, may also be mediated by JNK. In addition, regulation of the c-jun gene in p210 BCR-ABL transformed cells corresponds with activity of JNK, suggesting a role for JNK inhibitors in the treatment for chronic myelogenous leukemia (CML) [Blood 92:2450-60 (1998)].

JNK1 and JNK2 are widely expressed in a variety of tissues. In contrast, JNK3, is selectively expressed in the brain and to a lesser extent in the heart and testis [Gupta et al., *supra*; Mohit et al., Neuron 14:67-78 (1995); Martin et al., Brain Res. Mol. Brain Res. 35:47-57 (1996)]. JNK3 has been linked to neuronal apoptosis induced by kainic acid, indicating a role of JNK in the pathogenesis of glutamate neurotoxicity. In the adult human brain, JNK3 expression is localized to a subpopulation of pyramidal neurons in the CA1, CA4 and subiculum regions of the hippocampus and layers 3 and 5 of the neocortex [Mohit et al., *supra*]. The CA1 neurons of patients with acute hypoxia showed strong nuclear JNK3-immunoreactivity compared to minimal, diffuse cytoplasmic staining of the hippocampal neurons from brain tissues of normal patients [Zhang et al., *supra*]. Thus, JNK3 appears to be involved involved in hypoxic and ischemic damage of CA1 neurons in the hippocampus.

In addition, JNK3 co-localizes immunochemically with neurons vulnerable in Alzheimer's disease [Mohit et al., *supra*]. Disruption of the JNK3 gene caused resistance of mice to the excitotoxic glutamate receptor agonist kainic acid, including the effects on seizure

activity, AP-1 transcriptional activity and apoptosis of hippocampal neurons, indicating that the JNK3 signaling pathway is a critical component in the pathogenesis of glutamate neurotoxicity (Yang et al., Nature, 389:865-870 (1997)].

Based on these findings, JNK signalling, especially that of JNK3, has been implicated in the areas of apoptosis-driven neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, ALS (Amyotrophic Lateral Sclerosis), epilepsy and seizures, Huntington's Disease, traumatic brain injuries, as well as ischemic and hemorrhaging stroke.

There is a high unmet medical need to develop JNK specific inhibitors that are useful in treating the various conditions associated with JNK activation, especially considering the currently available, relatively inadequate treatment options for the majority of these conditions.

Recently, we have described crystallizable complexes of JNK protein and adenosine monophosphate, including complexes comprising JNK3, in U.S. Provisional Application 60/084056, filed May 4, 1998. Such information has been extremely useful in identifying and designing potential inhibitors of various members of the JNK family, which, in turn, have the described above therapeutic utility.

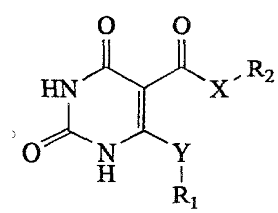
Much work has been done to identify and develop drugs that inhibit MAPKs, such as p38 inhibitors. See, e.g., WO 98/27098 and WO 95/31451. However, to our knowledge, no MAPK inhibitors have been shown to be specifically selective for JNKs versus other related MAPKs.

Accordingly, there is still a great need to develop potent inhibitors of JNKs, including JNK3 inhibitors, that are useful in treating various conditions associated with JNK activation.

## SUMMARY OF THE INVENTION

The present invention addresses this problem by providing compounds that demonstrate strong inhibition of JNK.

These compounds have the general formula:



(Formula I)

or pharmaceutically acceptable derivatives or prodrugs thereof.

Y is selected from O, NH, N(R), S, S(O) or $S(O)_2$.

X is selected from O, NH, or N(R).

$R^1$ and $R^2$ are each independently selected from H, a $C_1$-$C_6$ straight chain or branched alkyl or alkenyl group, optionally substituted with one to four substituents, each of which is independently selected from $NH_2$, NHR, $N(R)_2$, $NO_2$, OH, OR, $CF_3$, halo, CN, $CO_2H$, $CONH_2$, CONHR, $CON(R)_2$, COR, SR, S(O)R, $S(O)_2R$, $S(O)_2NH_2$, $S(O)_2NHR$ or R; a 5-7 membered aromatic or non-aromatic carbocyclic or heterocyclic ring, optionally substituted with one to four substituents, each of which is independently selected from $NH_2$, NHR, $N(R)_2$, $NO_2$, OH, OR, $CF_3$, halo, CN, $CO_2H$, $CONH_2$, CONHR, $CON(R)_2$, COR, SR, S(O)R, $S(O)_2R$, $S(O)_2NH_2$, $S(O)_2NHR$ or R; or a 9-10 membered bicyclic aromatic or non-aromatic carbocyclic or heterocyclic ring optionally substituted

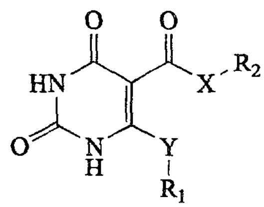
with one to four substituents, each of which is independently selected from $NH_2$, NHR, $N(R)_2$, $NO_2$, OH, OR, $CF_3$, halo, CN, $CO_2H$, $CONH_2$, CONHR, $CON(R)_2$, COR, SR, S(O)R, $S(O)_2R$, $S(O)_2NH_2$, $S(O)_2NHR$ or R.

R is a $C_1$-$C_6$ straight chain or branched alkyl or alkenyl group, a 5-7 membered aromatic or non-aromatic carbocyclic or heterocyclic ring, or a 9-10 membered bicyclic aromatic or non-aromatic carbocyclic or heterocyclic ring system.

In another embodiment, the invention provides pharmaceutical compositions comprising the JNK inhibitors of this invention. These compositions may be utilized in methods for treating or preventing a variety of disorders, such as heart disease, immunodeficiency disorders, inflammatory diseases, allergic diseases, autoimmune diseases, destructive bone disorders such as osteoporosis, proliferative disorders, infectious diseases and viral diseases. These compositions are also useful in methods for preventing cell death and hyperplasia and therefore may be used to treat or prevent reperfusion/ischemia in stroke, heart attacks, and organ hypoxia. The compositions are also useful in methods for preventing thrombin-induced platelet aggregation. The compositions are especially useful for disorders such as chronic myelogenous leukemia (CML), rheumatoid arthritis, asthma, osteoarthritis, ischemia, cancer, liver disease including hepatic ischemia, heart disease such as myocardial infarction and congestive heart failure, pathologic immune conditions involving T cell activation and neurodegenerative disorders. Each of these above-described methods is also part of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

The JNK inhibitors of the invention have the general formula:



(Formula I)

or pharmaceutically acceptable derivatives or prodrugs thereof.

Y is selected from O, NH, N(R), S, S(O) or $S(O)_2$.

X is selected from O, NH or N(R).

$R^1$ and $R^2$ are each independently selected from H, a $C_1$-$C_6$ straight chain or branched alkyl or alkenyl group, optionally substituted with one to four substituents, each of which is independently selected from $NH_2$, NHR, $N(R)_2$, $NO_2$, OH, OR, $CF_3$, halo, CN, $CO_2H$, $CONH_2$, CONHR, $CON(R)_2$, COR, SR, S(O)R, $S(O)_2R$, $S(O)_2NH_2$, $S(O)_2NHR$ or R; a 5-7 membered aromatic or non-aromatic carbocyclic or heterocyclic ring, optionally substituted with one to four substituents, each of which is independently selected from $NH_2$, NHR, $N(R)_2$, $NO_2$, OH, OR, $CF_3$, halo, CN, $CO_2H$, $CONH_2$, CONHR, $CON(R)_2$, COR, SR, S(O)R, $S(O)_2R$, $S(O)_2NH_2$, $S(O)_2NHR$ or R; or a 9-10 membered bicyclic aromatic or non-aromatic carbocyclic or heterocyclic ring optionally substituted with one to four substituents, each of which is independently selected from $NH_2$, NHR, $N(R)_2$, $NO_2$, OH, OR, $CF_3$, halo, CN, $CO_2H$, $CONH_2$, CONHR, $CON(R)_2$, COR, SR, S(O)R, $S(O)_2R$, $S(O)_2NH_2$, $S(O)_2NHR$ or R.

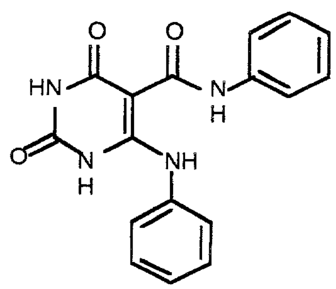
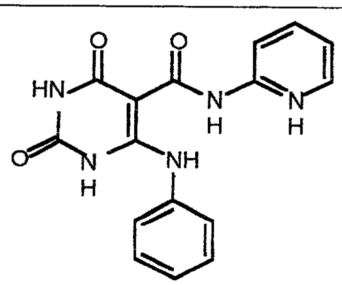
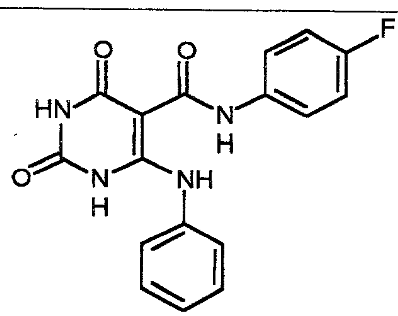
R is a $C_1$-$C_6$ straight chain or branched alkyl or alkenyl group, a 5-7 membered aromatic or non-aromatic carbocyclic or heterocyclic ring, or a 9-10 membered

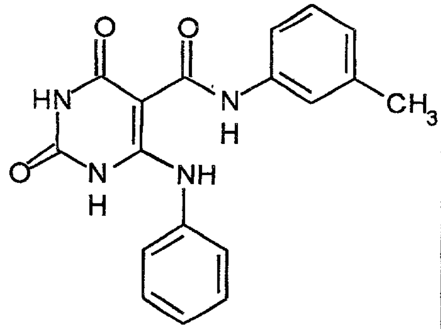
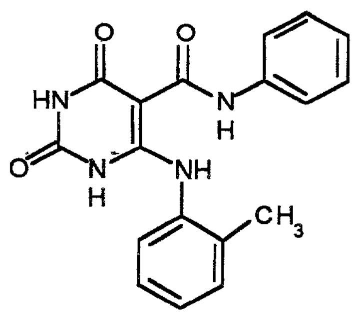
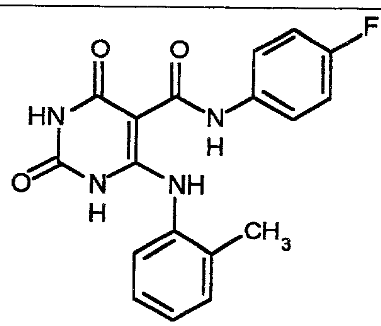
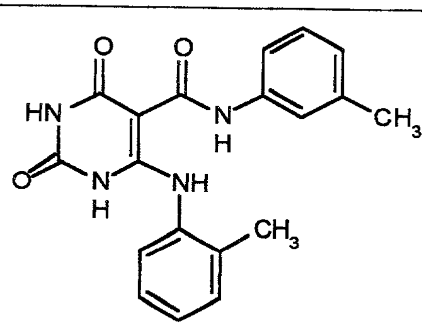
bicyclic aromatic or non-aromatic carbocyclic or heterocyclic ring system.

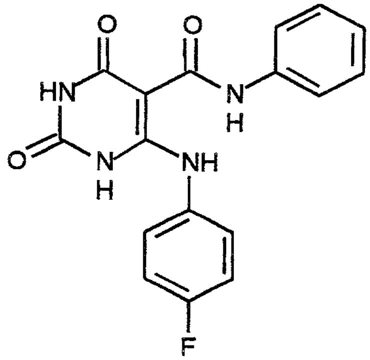
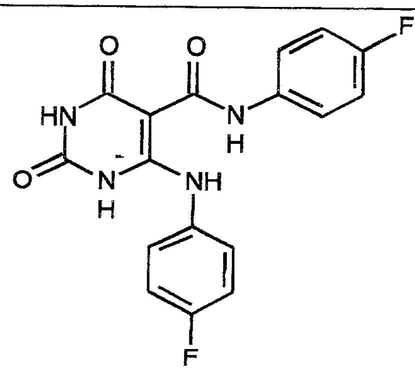
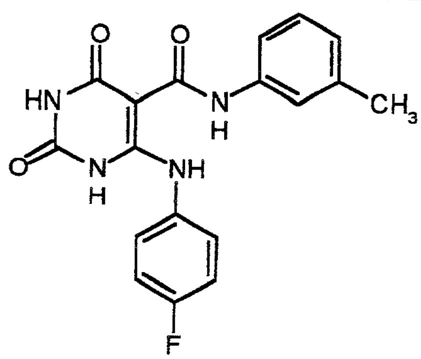
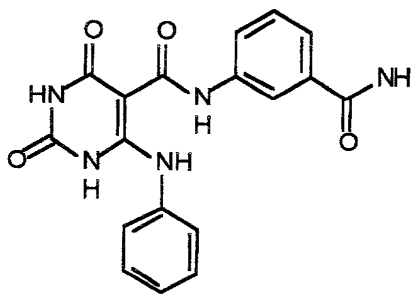
A heterocyclic ring system or a heterocyclic ring as defined herein is one that contains 1 to 4 heteroatoms, which are independently selected from N, O, S, SO and $SO_2$.

Some specific examples of preferred compounds of the instant invention are provided in Table 1 below. In Table 1, "+" represents a Ki $\geq$ 1 μM, "++" represents a Ki < 1 μM, and "ND" means not determined. The Ki is determined by the method disclosed in Example 3.

Table 1

| Cmpd | Structure | Ki |
|---|---|---|
| 1 |  | ++ |
| 2 |  | + |
| 3 |  | + |

| Cmpd | Structure | Ki |
|---|---|---|
| 4 |  | + |
| 5 |  | + |
| 6 |  | + |
| 7 |  | + |

| Cmpd | Structure | Ki |
|---|---|---|
| 8 |  | + |
| 9 |  | + |
| 10 |  | + |
| 11 |  | ND |

According to another embodiment, the present invention provides methods of producing JNK inhibitors of Formula I. Synthesis schemes for these compounds are described in Examples 1 and 2.

According to another embodiment of the invention, the activity of the JNK inhibitors of this invention may be assayed *in vitro, in vivo* or in a cell line. *In vitro* assays include assays that determine inhibition of either the kinase activity or ATPase activity of activated JNK. For example, see Examples 3 to 5. Alternate *in vitro* assays quantitate the ability of the inhibitor to bind to JNK and may be measured either by radiolabelling the inhibitor prior to binding, isolating the inhibitor/JNK complex and determining the amount of radiolabel bound, or by running a competition experiment where new inhibitors are incubated with JNK bound to known radioligands. One may use any type or isoform of JNK, depending upon which JNK type or isoform is to be inhibited.

The JNK inhibitors or pharmaceutical salts thereof may be formulated into pharmaceutical compositions for administration to animals or humans. These pharmaceutical compositions, which comprise an amount of JNK inhibitor effective to treat or prevent a JNK-mediated condition and a pharmaceutically acceptable carrier, are another embodiment of the present invention.

The term "JNK-mediated condition", as used herein means any disease or other deleterious condition in which JNK is known to play a role. Such conditions include, without limitation, inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, cancer, infectious diseases,

neurodegenerative diseases, allergies, reperfusion/ischemia in stroke, heart attacks, angiogenic disorders, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, thrombin-induced platelet aggregation, and conditions associated with prostaglandin endoperoxidase synthase-2.

Inflammatory diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, acute pancreatitis, chronic pancreatitis, asthma, allergies, and adult respiratory distress syndrome.

Autoimmune diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, atopic dermatitis, chronic active hepatitis, myasthenia gravis, multiple sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, psoriasis, or graft vs. host disease.

Destructive bone disorders which may be treated or prevented by the compounds of this invention include, but are not limited to, osteoporosis, osteoarthritis and multiple myeloma-related bone disorder.

Proliferative diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma and HTLV-1 mediated tumorigenesis.

Angiogenic disorders which may be treated or prevented by the compounds of this invention include solid tumors, ocular neovasculization, infantile haemangiomas. Infectious diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, sepsis, septic shock, and Shigellosis.

Viral diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, acute hepatitis infection (including hepatitis A, hepatitis B and hepatitis C), HIV infection and CMV retinitis.

Neurodegenerative diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), epilepsy, seizures, Huntington's disease, traumatic brain injury, ischemic and hemorrhaging stroke, cerebral ischemias or neurodegenerative disease, including apoptosis-driven neurodegenerative disease, caused by traumatic injury, acute hypoxia, ischemia or glutamate neurotoxicity.

"JNK-mediated conditions" also include ischemia/reperfusion in stroke, heart attacks, myocardial ischemia, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, hepatic ischemia, liver disease, congestive heart failure, pathologic immune responses such as that caused by T cell activation and thrombin-induced platelet aggregation.

In addition, JNK inhibitors of the instant invention may be capable of inhibiting the expression of inducible pro-inflammatory proteins. Therefore, other "JNK-mediated conditions" which may be treated by the compounds of this invention include edema, analgesia,

fever and pain, such as neuromuscular pain, headache, cancer pain, dental pain and arthritis pain.

In addition to the compounds of this invention, pharmaceutically acceptable derivatives or prodrugs of the compounds of this invention may also be employed in compositions to treat or prevent the above-identified disorders.

A "pharmaceutically acceptable derivative or prodrug" means any pharmaceutically acceptable salt, ester, salt of an ester or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof. Particularly favored derivatives or prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species.

Pharmaceutically acceptable prodrugs of the compounds of this invention include, without limitation, esters, amino acid esters, phosphate esters, metal salts and sulfonate esters.

Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate,

camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium and potassium), alkaline earth metal (e.g., magnesium), ammonium and N-($C_{1-4}$ alkyl)$4^{+}$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate,

polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously.

Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their

polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic

uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

The amount of JNK inhibitor that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the particular mode of administration. Preferably, the compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of the inhibitor can be administered to a patient receiving these compositions.

It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of inhibitor will also depend upon the particular compound in the composition.

According to another embodiment, the invention provides methods for treating or preventing a JNK-mediated condition comprising the step of administering to a patient one of the above-described pharmaceutical

compositions. The term "patient", as used herein, means an animal, preferably a human.

Preferably, that method is used to treat or prevent a condition selected from inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, degenerative diseases, neurodegenerative diseases, allergies, reperfusion/ischemia in stroke, heart attacks, angiogenic disorders, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, and thrombin-induced platelet aggregation, or any specific disease or disorder described above.

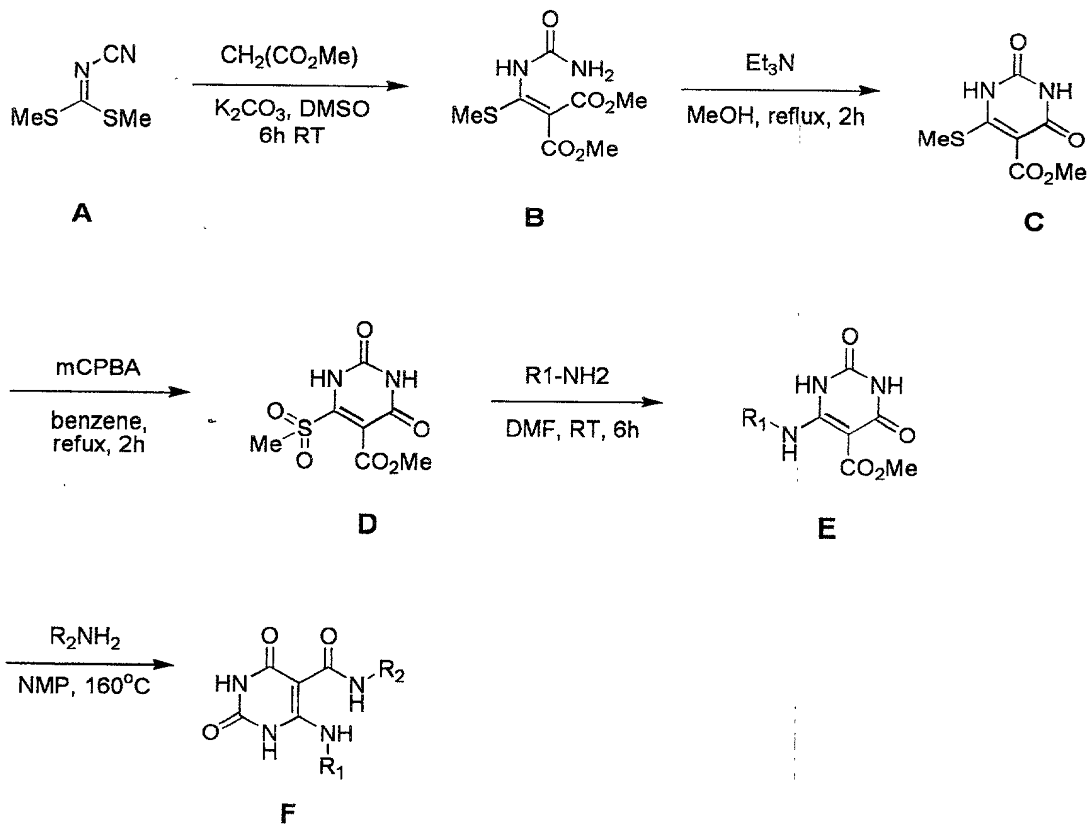
Depending upon the particular JNK-mediated condition to be treated or prevented, additional drugs, which are normally administered to treat or prevent that condition, may be administered together with the inhibitors of this invention. For example, chemotherapeutic agents or other anti-proliferative agents may be combined with the JNK inhibitors of this invention to treat proliferative diseases.

Those additional agents may be administered separately, as part of a multiple dosage regimen, from the JNK inhibitor-containing composition. Alternatively, those agents may be part of a single dosage form, mixed together with the JNK inhibitor in a single composition.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

## EXAMPLE 1

## General Synthesis of JNK Inhibitors of Formula I

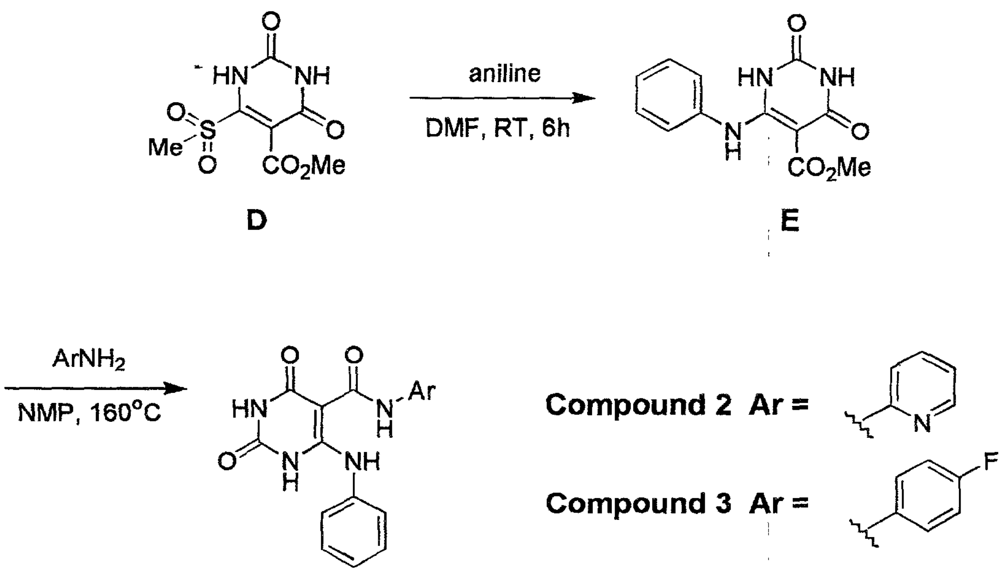


The general synthesis of compounds of Formula I wherein X and Y are amino groups is shown above. Compound **C** may be prepared as described by Tominaga, S. et al., J. Heterocyclic Chem., 28:1039-1042 (1991), or as described below in Example 2. In the general scheme, compound **C** is subsequently oxidized to the sulphone derivative **D** utilizing an oxidant such as meta-chloro-perbenzoic acid (mCPBA) or other oxidants known in the art. The intermediate **D** is then contacted with an amine to displace the sulphone moiety, thereby affording compounds of type **E**. Compound **E** is then contacted with a second amine to displace the methyl ester, thereby forming amides of type

**F**. Specific examples of type **F** compounds are described in Example 2. Alternatively, compound **E** may be saponified by contacting with a base such as sodium hydroxide, or lithium hydroxide to form an acid derivative. Such acid derivative could be coupled to various $R_1NH_2$ amines utilizing any one of numerous conditions known in the art to generate compounds of type **F**.

EXAMPLE 2

Synthesis of JNK Inhibitor Compounds 2 and 3




In a preferred embodiment of the general synthesis scheme outlined in Example 1, 20.1 g (137 mmol) dimethyl N-cyanodithioiminocarbonate (**A**) and 38.4 g (278 mmol) potassium carbonate ($K_2CO_3$) were dissolved in 180 mL dimethyl sulfoxide (DMSO). 23.5 mL (206 mmol) dimethylmalonate ($CH_2CO_2Me$) was added to the mixture. The reaction was stirred at room temperature for 6 hours (h), then poured into ice-cold aqueous 1.7M HCl. The solid precipitate was filtered, washed with water (500 mL) and dried. 2-(Methylsulfanyl-ureido-methylene)-malonic acid

dimethyl ester (**B**) was purified by thin layer chromatography (TLC) [Rf 0.45 (9:1 $CH_2C_{12}$:MeOH)]. The yield of **B** was 60%.

To prepare 6-methylsulfanyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid methyl ester (**C**), 24.55 g (98.9 mmol) compound **B** was suspended in 500 mL methanol (MeOH), and 22 mL (158 mmol) triethylamine ($Et_3N$) was added. The mixture was heated to reflux for 2 h. The solution was rotary evaporated to a viscous syrup, and methanol (~50 mL) and 3M HCl were added. The mixture was cooled and the solid was isolated by filtration. The product was washed with water (600 mL) and dried. The yield of **C** was 22.11 g.

5.39 g (24.9 mmol) **C** and 26.3 g m-chloro-peroxybenzoic acid (mCPBA) were heated to reflux in 150 mL benzene for 2 h. The solvent was then removed by rotary evaporation and the residue suspended in 70 mL ethyl acetate. The suspension was mixed for 40 minutes at room temperature, then filtered. The isolated solid was resuspended in 40 mL ethyl acetate, mixed for 40 minutes at room temperature, then filtered. The yield of 6-methanesulfonyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid methyl ester (**D**) was 53% (3.29 g).

To prepare 2,4-Dioxo-6-phenylamino-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid methyl ester (**E**), 214 mg (0.86 mmol) **D** was dissolved in 3 mL dimethyl formamide (DMF). 100 µL (1.1 mmol) aniline was added and the reaction stirred at room temperature for 6 h. The reaction was cooled to room temperature and the precipitate was filtered, washed with approximately 1.5 mL DMF and 5 mL diethyl ether, then dried. The yield of **E** was 86 mg (38%).

Compound **E** was synthesized from **D** as described above in Example 1. 21.7 mg (83 μmol) **E** was added to 250 μL N-methyl pyrrolidinone (NMP) and 106 mg (1.13 mmol) 2-aminopyridine. The reaction was heated at 160°C for 5 h, then cooled. 3 mL 3M HCl was added to the cool mixture and the solution of the crude product 2,4-Dioxo-6-phenylamino-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid pyridin-2-ylamide (**2**) was directly purified by preparative HPLC.

To prepare 2,4-dioxo-6-phenylamino-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid (4-fluoro-phenyl)-amide (**3**), 250 μL NMP and 250 μL 4-fluoroaniline was added to 22.8 mg (87 μmol) **E**. The reaction was heated at 160°C for 5 h, then cooled. 5 mL 3M HCl was added to the cool mixture and the precipitate was isolated by filtration. The crude product of **3** was dissolved in aqueous acetonitrile and purified by preparative HPLC.

## EXAMPLE 3

## Cloning, Expression and Purification of JNK3 Protein

A BLAST search of the EST database using the published JNK3α1 cDNA as a query identified an EST clone (#632588) that contained the entire coding sequence for human JNK3α1. Polymerase chain reactions (PCR) using *pfu* polymerase (Strategene) were used to introduce restriction sites into the cDNA for cloning into the pET-15B expression vector at the NcoI and BamHI sites. The protein was expressed in *E. coli*. Due to the poor solubility of the expressed full-length protein (Met 1-Gln 422), an N-terminally truncated protein starting at Ser residue at position 40 (Ser 40) was produced. This truncation corresponds to Ser 2 of JNK1 and JNK2 proteins, and is preceded by a methionine (initiation) and a glycine

residue. The glycine residue was added in order to introduce an NcoI site for cloning into the expression vector. In addition, systematic C-terminal truncations were performed by PCR to identify a construct that give rise to diffraction-quality crystals. One such construct encodes amino acid residues Ser40-Glu402 of JNK3α1 and is preceded by Met and Gly residues.

The construct was prepared by PCR using deoxyoligonucleotides

5' GCTCTAGAGCTCCATGGGCAGCAAAAGCAAAGTTGACAA 3' (forward primer with initiation codon underlined) and

5' TAGCGGATCCTCATTCTGAATTCATTACTTCCTTGTA 3' (reverse primer with stop codon underlined)

as primers and was confirmed by DNA sequencing. Control experiments indicated that the truncated JNK3 protein had an equivalent kinase activity towards myelin basic protein when activated with an upstream kinase MKK7 *in vitro*.

*E.coli* strain BL21 (DE3) (Novagen) was transformed with the JNK3 expression construct and grown at 30°C in LB supplemented with 100 μg/ml carbenicillin in shaker flasks until the cells were in log phase ($OD_{600}$ ~ 0.8). Isopropylthio-β-D-galactosidase (IPTG) was added to a final concentration of 0.8 mM and the cells were harvested 2 hours later by centrifugation.

*E. coli* cell paste containing JNK3 was resuspended in 10 volumes/g lysis buffer (50 mM HEPES, pH 7.2, containing 10% glycerol (v/v), 100 mM NaCl, 2 mM DTT, 0.1 mM PMSF, 2 μg/ml Pepstatin, 1μg/ml each of E-64 and Leupeptin). Cells were lysed on ice using a microfluidizer and centrifuged at 100,000 x *g* for 30 min at 4 °C. The 100,000 x *g* supernatant was diluted 1:5 with Buffer A (20 mM HEPES, pH 7.0, 10% glycerol (v/v), 2 mM

DTT) and purified by SP-Sepharose (Pharmacia) cation-exchange chromatography (column dimensions: 2.6 x 20 cm) at 4 $^{0}$C. The resin was washed with 5 column volumes of Buffer A, followed by 5 column volumes of Buffer A containing 50 mM NaCl. Bound JNK3 was eluted with a 7.5 column volume linear gradient of 50-300 mM NaCl. JNK3 eluted between 150-200 mM NaCl.

## EXAMPLE 4

## Activation of JNK3

5 mg of JNK3 was diluted to 0.5 mg/ml in 50 mM HEPES buffer, pH 7.5, containing 100 mM NaCl, 5 mM DTT, 20 mM $MgCl_2$ and 1 mM ATP. GST-MKK7(DD) was added at a molar ratio of 1:2.5 GST-MKK7:JNK3. After incubation for 30 minutes at 25°C, the reaction mixture was concentrated 5-fold by ultrafiltration in a Centriprep-30 (Amicon, Beverly, MA), diluted to 10 ml and an additional 1 mM ATP added. This procedure was repeated three times to remove ADP and replenish ATP. The final addition of ATP was 5 mM and the mixture incubated overnight at 4°C.

The activated JNK3/GST-MKK7(DD) reaction mixture was exchanged into 50 mM HEPES buffer, pH 7.5, containing 5 mM DTT and 5% glycerol (w/v) by dialysis or ultrafiltration. The reaction mixture was adjusted to 1.1 M potassium phosphate, pH 7.5, and purified by hydrophobic interaction chromatography (at 25 °C) using a Rainin Hydropore column. GST-MKK7 and unactivated JNK3 do not bind under these conditions such that when a 1.1 to 0.05 M potassium phosphate gradient is developed over 60 minutes at a flow rate of 1 ml/minute, doubly phosphorylated JNK3 is separated from singly phosphorylated JNK. Activated

JNK3 (i.e. doubly phosphorylated JNK3) was stored at $-70^{0}C$ at 0.25-1 mg/ml.

## EXAMPLE 5

## JNK Inhibition Assays

Compounds were assayed for the inhibition of JNK3 by a spectrophotometric coupled-enzyme assay. In this assay, a fixed concentration of activated JNK3 (10 nM) was incubated with various concentrations of a potential inhibitor dissolved in DMSO for 10 minutes at 30°C in a buffer containing 0.1 M HEPES buffer, pH 7.5, containing 10 mM $MgCl_2$, 2.5 mM phosphoenolpyruvate, 200 µM NADH, 150 µg/mL pyruvate kinase, 50 µg/mL lactate dehydrogenase, and 200 µM EGF receptor peptide. The EGF receptor peptide has the sequence KRELVEPLTPSGEAPNQALLR, and is a phosphoryl acceptor in the JNK3-catalyzed kinase reaction. The reaction was initiated by the addition of 10 µM ATP and the assay plate is inserted into the spectrophotometer's assay plate compartment that was maintained at 30°C. The decrease of absorbance at 340 nm was monitored as a function of time. The rate data as a function of inhibitor concentration was fitted to competitive inhibition kinetic model to determine the $K_i$.